NUCLEAR MAGNETIC RESONANCE METHODS FOR IDENTIFYING SITES IN PAPILLOMAVIRUS E2 PROTEIN

This application claims the benefit of U.S. Provisional Application Serial Nos. 60/197,459, filed 17 April 2000, 60/211,055, filed 13 June 2000, and 60/268,444 filed 13 February 2001, which are incorporated herein by reference in their entireties.

BACKGROUND OF THE INVENTION

An important aspect in understanding the function of biochemical processes is the elucidation of the nature of the associations between various species including, for example, the associations between ligands and proteins. Such associations may be non-covalent, wherein juxtapositions are energetically favored by hydrogen bonding, van der Waals forces, or electrostatic interactions, or they may be covalent. When physical binding is being studied, a target molecule is typically exposed to one or more compounds suspected of being ligands, and assays are then performed to determine if complexes between the target molecule and one or more of those compounds are formed. Such assays, as are well known in the art, test for gross changes (e.g., size, charge, and mobility) in the target molecule that indicate complex formation.

Where functional changes are measured, assay conditions are established that allow for measurement of biological or chemical events related to the target molecule (e.g., enzyme catalyzed reaction and receptor-mediated enzyme activation). To identify an alteration, the function of the target molecule is determined before and after exposure to the test compounds.

Assays involving the use of nuclear magnetic resonance (NMR) techniques are also known. NMR techniques may be used, for example, in conjunction with other assay methods to assess hits identified from physical binding screens or functional assay screens. If ^{1}H, ^{13}C, and/or ^{15}N resonance assignments are known for the target as well as either a solution or X-ray crystallographic structure, then the binding site location of identified ligands can be determined using NMR techniques.

As such, definitive resonance assignments of the target are required as a first step. A DNA-binding protein, E2, which is encoded by the papillomavirus and is involved in transcriptional regulation and viral replication, is one such target.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a nuclear magnetic resonance method for identifying a site in a DNA-binding and dimerization domain of a papillomavirus E2 protein. In one embodiment, the method includes providing a first set of chemical shifts for atoms of a mixture including a ligand and the papillomavirus E2 protein, comparing the first set of chemical shifts to a second set of chemical shifts as listed in Table 1, and identifying at least a portion of the atoms that exhibit changes in chemical shifts, wherein the site includes the identified atoms. Preferably providing the first set of chemical shifts includes providing a mixture of the ligand and the papillomavirus E2 protein, allowing the ligand to interact with the papillomavirus E2 protein, obtaining a nuclear magnetic resonance spectrum of the mixture, and measuring chemical shifts of atoms from the spectrum. Preferably allowing the ligand to interact includes allowing the ligand and the protein to reach a binding equilibrium. Preferably the site is a ligand binding site. Preferably the papillomavirus E2 protein is encoded by the HPV-18 strain.

In another embodiment, the method includes providing a first ^{1}H-^{15}N heteronuclear single quantum correlation spectrum of a mixture including a ligand and the papillomavirus E2 protein, comparing the first ^{1}H-^{15}N heteronuclear single quantum correlation spectrum to a second ^{1}H-^{15}N heteronuclear single quantum correlation spectrum as illustrated in Figure 2, and identifying at least a portion of the amino acids having atoms that exhibit changes in chemical shifts, wherein the site includes the identified amino acids. Preferably providing the first spectrum includes providing a mixture of the ligand and the papillomavirus E2 protein, allowing the ligand to interact with the papillomavirus E2 protein, and obtaining a ^{1}H-^{15}N heteronuclear single quantum correlation spectrum of the mixture. Preferably allowing the ligand to interact includes allowing the ligand and the

protein to reach a binding equilibrium. Preferably the site is a ligand binding site. Preferably the papillomavirus E2 protein is encoded by the HPV-18 strain.

In another aspect, the present invention provides a machine-readable data storage medium including a data storage material encoded with nuclear magnetic resonance chemical shifts as listed in Table 1, wherein when a first set of chemical shifts is provided, the chemical shifts encoded on the data storage material are capable of being read by the machine to create a second set of chemical shifts, and the machine having programmed instructions that are capable of causing the machine to compare the first and second sets of chemical shifts to arrive at structural information.

In another aspect, the present invention provides a computer-assisted method for identifying a ligand binding site in a DNA-binding and dimerization domain of a papillomavirus E2 protein. The method includes providing a first set of nuclear magnetic resonance chemical shifts for atoms of a mixture including the ligand and the papillomavirus E2 protein, causing the first set of chemical shifts to be entered into memory of a computer, causing the computer to read a second set of chemical shifts as listed in Table 1 from a machine-readable data storage medium, causing the computer to compare the first and second sets of chemical shifts, and causing the computer to identify at least a portion of the atoms that exhibit changes in chemical shifts, wherein the ligand binding site includes the identified atoms. Preferably the papillomavirus E2 protein is encoded by the HPV-18 strain. Preferably the method further includes causing the computer to visually display a spatial arrangement of atoms of the ligand binding site.

Methods disclosed in the present invention for identifying sites offer advantages over other methods known in the art. For example, the present invention preferably provides methods for efficiently identifying binding sites for a wide range of chemically and physically diverse potential ligands.

The term "binding" as used herein, refers to a condition of proximity between a chemical entity or compound, or portions thereof, and the target protein or portions thereof. The association may be non-covalent, wherein the juxtaposition

is energetically favored by hydrogen bonding, van der Waals forces, or electrostatic interactions, or it may be covalent. The association may be a static interaction, or an equilibrium may be reached between associated and non-associated species. Preferably, a ligand that binds to a ligand binding site in a DNA-binding and dimerization domain of a papillomavirus E2 protein would also be expected to bind to or interfere with another ligand binding site whose structure defines a shape that falls within an acceptable error.

The term "ligand" as used herein means any chemical entity, compound, or portion thereof, that is capable of binding to a protein.

The term "change in chemical shifts" as used herein means the observation of an increase or decrease in chemical shift for a resonance, an increase or decrease in intensity for a resonance, or the failure to observe a resonance when comparing a resonance of an atom from the spectrum of a mixture of ligand and protein to the resonance of the same atom from the spectrum of the protein without the ligand

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an illustration of the deviations from random coil chemical shifts of $^{13}C_{\alpha}$ resonances (in parts per million (ppm)) with assignments for the DNA-binding and dimerization domain of papillomavirus (strain HPV-18) E2 protein as a function of residue number. Random coil chemical shift values are from Wishart et al., Biochem. Cell Biol., 76:153-63 (1998). Locations of secondary structure according to the X-ray structure of BPV-1, HPV-16 and HPV-31 are shown with α (α-helix) and β (β-sheet).

Figure 2 is an illustration of the 2-dimensional ^{1}H-^{15}N heteronuclear single quantum correlation spectrum with assignments for the DNA-binding and dimerization domain of a 0.84 mM papillomavirus (strain HPV-18) E2 protein at 300°K.

DETAILED DESCRIPTION

Papillomaviruses are a diverse group of small DNA viruses that infect epithelial cells and cause tumor formation. All of the papillomaviruses encode a DNA-binding protein, E2, that is involved in transcriptional regulation and viral replication. E2 protein consists of a C-terminal DNA-binding and dimerization domain (E2-DBD) and N-terminal transactivation domain, separated by a flexible region. E2-DBD from bovine papillomavirus-1 (BPV-1) has been extensively studied, and the X-ray crystallographic structure of E2-DBD bound to DNA consists of a homodimer that includes an eight-stranded β-barrel and two pairs of α-helices (Hedge et al., Nature, 359:505-12 (1992)). The solution and/or crystal structures of homologous E2-DBDs from human papillomavirus-31 (HPV-31) (Liang et al., Biochemistry, 35:2095-2103 (1996), Bussiere et al., Acta Cryst., D54:1367-76 (1998)) and HPV-16 (Hedge et al., J. Mol. Biol., 284:1479-89 (1998)) have been reported and are similar to BPV-1.

The present invention preferably relates to the E2-DBD from the high risk strain HPV-18. The E2 protein of HPV-18 represses the expression of the major viral transforming genes E6 and E7 and is a cofactor for the replication protein E1 binding to the origin (Kasukawa et al., J. Virol., 72:8166-73 (1998)). The pivotal role of E2 in transcriptional regulation and viral replication makes it a potential target for antiviral therapy.

E2-DBD of HPV-18 has 55% and 60% sequence identity to HPV-16 and HPV-31, respectively, and binds to the $ACCN_6GGT$ recognition sequence. Preferably, two amino acid sequences are compared using the Blastp program, version 2.0.9, of the BLAST 2 search algorithm, as described by Tatusova et al., FEMS Microbiol Lett 174, 247-50 (1999), and available at http://www.ncbi.nlm.nih.gov/gorf/bl2.html. Preferably, the default values for all BLAST 2 search parameters are used, including matrix = BLOSUM62; open gap penalty = 11, extension gap penalty = 1, gap x_dropoff = 50, expect = 10, wordsize = 3, and filter on. In the comparison of two amino acid sequences using the BLAST search algorithm, structural similarity is referred to as "identity."

The present invention provides a papillomavirus HPV-18 strain E2 protein DNA-binding domain having the ^{1}H-^{15}N heteronuclear single quantum correlation spectrum shown in Figure 2. Each correlation is labeled as to the residue in the protein from which it arises if that has been determined. The process used to make the assignments is described in the examples. The chemical shifts of all assigned ^{1}H, ^{13}C, and ^{15}N resonances are listed in Table 1. The resonance assignments presented here provide the basis for determining sites, preferably binding site locations of ligands previously identified by other means. Chemical shift changes induced by addition of ligand to the protein sample are manifested by changes in the appearance of ^{1}H-^{15}N HSQC spectra. Correlations that experience the largest ligand-induced chemical shift changes are preferably located near the ligand's binding site. To determine chemical shift changes, the protein ^{1}H, ^{13}C, and ^{15}N resonances are preferably assigned as extensively as possible.

Preferably, ligand binding sites include identified atoms that exhibit changes in chemical shifts. Preferably the identified atoms include at least one proton that, upon addition of ligand to the protein, either exhibits a change in ^{1}H chemical shift of at least about 0.04 ppm or is no longer observed. Preferably the identified atoms includes at least one carbon atom that, upon addition of ligand to the protein, either exhibits a change in ^{13}C chemical shift of at least about 0.2 ppm or is no longer observed. Preferably the identified atoms include at least one nitrogen atom that, upon addition of ligand to the protein, either exhibits a change in ^{15}N chemical shift of at least about 0.2 ppm or is no longer observed.

In order that this invention be more fully understood, the following examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

EXAMPLES

The HPV-18 E2 protein consists of 410 amino acids with the DBD residing at the C-terminus (amino acids #329-410). E2-DBD cloning procedures resulted in the addition of methionine before amino acid 329 and six histidine residues after

amino acid 410. Amino acid sequencing indicated that the N-terminal des-Met form of the E2-DBD protein was the major species produced.

E2-DBD was over-expressed in BL21 (DE3) *E. coli* cells using the pSRtac vector. Isotopically labeled samples were prepared in M9 glucose media containing $^{15}NH_4Cl$ and unlabeled or U-^{13}C-glucose. Cell pellets were lysed with intermittent mechanical disruption with a Tissuemizer (Tekmar Co., Cincinatti, OH). Clarified cell lysates were passed over Ni^{2+}-NTA agarose (Qiagen, Inc., Valencia, CA), and further purified using Source 30Q anion exchange chromatography (Amersham Pharmacia Biotech, Inc.; Piscataway, NJ). The resulting E2-DBD exists as a homodimer of molecular weight 20.6 kDa under the conditions used for the NMR experiments.

The NMR samples typically consisted of 0.8 mM protein in buffer containing 20 mM phosphate, 50 mM NaCl, and 1 mM [$^2H_{10}$] dithiothreitol (DTT) at pH 6.5 in 90% ^1H_2O/10% ^2H_2O by volume. All NMR spectra were recorded at 27°C on a Bruker DRX-600 spectrometer (BRUKER NMR, Rheinstetten, Germany) using a 5 mm triple-resonance probe with 3-axis gradients. HNC_α, $HN(CO)C_\alpha$, $C_\beta C_\alpha(CO)NH$, $H_\beta H_\alpha(CO)NH$, HNCO and HCCH-total correlation spectroscopy (HCCH-TOCSY) (mixing times 16 and 23 milliseconds) data sets were acquired using gradient-enhanced versions of the pulse sequences. Two-dimensional ^{1}H-^{15}N Heteronuclear Single Quantum Correlation (HSQC) and ^{15}N edited Nuclear Overhauser Effect Spectroscopy-HSQC (NOESY-HSQC) (mixing time 80 milliseconds) spectra were also acquired. Proton chemical shifts were referenced to the ^1H_2O signal at 4.70 parts per million (ppm) (tetramethylsilane (TMS) = 0 ppm). The ^{15}N and ^{13}C chemical shifts were referenced indirectly in a manner similar to that known in the art (e.g., Bax et al., J. Magn. Reson., 67:565-69 (1986)). Carrier frequencies were 4.70 ppm for ^{1}H, 118 ppm for ^{15}N, 54 ppm for $^{13}C_\alpha$, 40 ppm for aliphatic ^{13}C, and 174 ppm for ^{13}C'. A combination of water flip-back (e.g., Grzesiek et al., J. Am. Chem. Soc., 115:12593-94 (1993)) and WATERGATE (e.g., Piotto et al., J. Biomol. NMR, 2:661-65 (1992)) techniques were used to eliminate

the water resonance. NMR data were processed using NMRPipe and NMRDraw software from Molecular Simulations, Inc. (San Diego, CA).

Sequence-specific backbone resonance assignments were accomplished using primarily 3-dimensional HNC_α, $HN(CO)C_\alpha$, and $C_\beta C_\alpha(CO)NH$ data sets. The $^{13}C'$ and $^1H_\alpha$, $^1H_\beta$ chemical shifts were determined using HNCO and $H_\beta H_\alpha(CO)NH$ data sets, respectively. The side chain ^1H and ^{13}C spin systems were assigned using the 3-dimensional HCCH-TOCSY experiments.

The assigned ^1H-^{15}N HSQC spectrum of HPV-18 E2-DBD is shown in Figure 2. Chemical shift values for all ^1H_N, $^1H_\alpha$, $^{13}C_\alpha$, $^{13}C_\beta$, $^{13}C'$ and $^{15}N_\alpha$ resonances except for the first four residues, the C-terminal five histidine residues, and Glu58 and Thr59 were assigned. Approximately 60% of the side chain ^1H and ^{13}C resonances were also assigned. Assigned ^1H, ^{13}C, and ^{15}N chemical shifts are listed in Table 1. The locations of secondary structure in the linear amino acid sequence predicted based on $^{13}C_\alpha$ chemical shifts (see Wishart et al., J. Biomol. NMR, 4:171-80 (1994)) are shown in Figure 1 and are consistent with the crystal structures of BPV-1, HPV-16 and HPV-31.

The complete disclosure of all patents, patent applications, and publications, and electronically available material cited herein are incorporated by reference. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

Table 1: ^{1}H, ^{13}C, and ^{15}N chemical shifts of human papillomavirus E2-DBD. HA, HB, HG, HD, HE, CA, CB, CG, CD, CE refer to H_α, H_β, H_γ, H_δ, H_ε, C_α, C_β, C_γ, C_δ, and C_ε respectively.

#Atom	#RES	RES	ATOMS		ppm
1	4	THR	HA	H	5.01
2	4	THR	HB	H	3.91
3	4	THR	HG1	H	0.98
4	4	THR	HG2	H	0.98
5	4	THR	CA	C	59.95
6	4	THR	CB	C	67.75
7	4	THR	CG2	C	19.93
8	5	THR	H	H	9.18
9	5	THR	C	C	171.68
10	5	THR	CA	C	57.48
11	5	THR	N	N	124.16
12	6	PRO	HA	H	4.73
13	6	PRO	CA	C	60.10
14	6	PRO	CB	C	29.24
15	7	ILE	H	H	8.49
16	7	ILE	HA	H	5.85
17	7	ILE	HB	H	1.82
18	7	ILE	HG2	H	0.92
19	7	ILE	HD1	H	0.49
20	7	ILE	C	C	173.65
21	7	ILE	CA	C	57.29
22	7	ILE	CB	C	42.10
23	7	ILE	CG2	C	16.79
24	7	ILE	CD1	C	12.90
25	7	ILE	N	N	115.39
26	8	ILE	H	H	8.90
27	8	ILE	HA	H	5.01
28	8	ILE	HB	H	1.88
29	8	ILE	HG2	H	0.82
30	8	ILE	C	C	174.83
31	8	ILE	CA	C	58.93
32	8	ILE	CB	C	39.92
33	8	ILE	CG2	C	15.73
34	8	ILE	N	N	115.93
35	9	HIS	H	H	8.91
36	9	HIS	HA	H	5.68
37	9	HIS	HB2	H	2.81
38	9	HIS	HB3	H	2.57
39	9	HIS	C	C	173.19
40	9	HIS	CA	C	51.27
41	9	HIS	CB	C	32.38
42	9	HIS	N	N	119.91
43	10	LEU	H	H	8.98
44	10	LEU	HA	H	5.17
45	10	LEU	HB2	H	1.66
46	10	LEU	HB3	H	0.92
47	10	LEU	HG	H	1.47
48	10	LEU	HD1	H	0.82
49	10	LEU	HD2	H	0.71
50	10	LEU	C	C	172.40
51	10	LEU	CA	C	50.25
52	10	LEU	CB	C	40.76
53	10	LEU	CG	C	23.68

54	10	LEU	N	N	122.16
55	11	LYS	H	H	8.76
56	11	LYS	HA	H	5.29
57	11	LYS	HB2	H	1.65
58	11	LYS	HB3	H	1.44
59	11	LYS	HG2	H	1.40
60	11	LYS	HG3	H	1.21
61	11	LYS	HD2	H	1.62
62	11	LYS	HD3	H	1.62
63	11	LYS	HE2	H	2.70
64	11	LYS	HE3	H	2.70
65	11	LYS	C	C	172.59
66	11	LYS	CA	C	51.76
67	11	LYS	CB	C	33.58
68	11	LYS	CG	C	22.68
69	11	LYS	CD	C	27.38
70	11	LYS	CE	C	39.54
71	11	LYS	N	N	120.73
72	12	GLY	H	H	8.30
73	12	GLY	HA2	H	4.43
74	12	GLY	HA3	H	4.19
75	12	GLY	C	C	173.46
76	12	GLY	CA	C	42.96
77	12	GLY	N	N	109.97
78	13	ASP	H	H	8.50
79	13	ASP	HA	H	4.59
80	13	ASP	HB2	H	2.77
81	13	ASP	HB3	H	2.61
82	13	ASP	C	C	168.61
83	13	ASP	CA	C	52.23
84	13	ASP	CB	C	40.03
85	13	ASP	N	N	120.16
86	14	ARG	H	H	8.61
87	14	ARG	HA	H	3.58
88	14	ARG	HB2	H	1.72
89	14	ARG	HB3	H	1.68
90	14	ARG	HG2	H	1.47
91	14	ARG	HG3	H	1.47
92	14	ARG	HD2	H	3.07
93	14	ARG	HD3	H	3.02
94	14	ARG	C	C	174.68
95	14	ARG	CA	C	58.64
96	14	ARG	CB	C	27.87
97	14	ARG	CG	C	26.01
98	14	ARG	CD	C	40.85
99	14	ARG	N	N	122.34
100	15	ASN	H	H	8.64
101	15	ASN	HA	H	4.46
102	15	ASN	HB2	H	2.87
103	15	ASN	HB3	H	2.76
104	15	ASN	C	C	176.39
105	15	ASN	CA	C	54.42
106	15	ASN	CB	C	35.59
107	15	ASN	N	N	118.46
108	16	SER	H	H	8.35
109	16	SER	HA	H	3.86
110	16	SER	HB2	H	4.17
111	16	SER	HB3	H	3.63
112	16	SER	C	C	175.96
113	16	SER	CA	C	59.80
114	16	SER	CB	C	59.96

115	16	SER	N	N	118.74
116	17	LEU	H	H	8.10
117	17	LEU	HA	H	3.84
118	17	LEU	HB2	H	1.64
119	17	LEU	HB3	H	1.17
120	17	LEU	HD1	H	0.45
121	17	LEU	HD2	H	0.38
122	17	LEU	C	C	175.25
123	17	LEU	CA	C	55.37
124	17	LEU	CB	C	38.75
125	17	LEU	CD1	C	23.04
126	17	LEU	CD2	C	19.79
127	17	LEU	N	N	121.15
128	18	LYS	H	H	7.83
129	18	LYS	HA	H	3.91
130	18	LYS	HB2	H	1.97
131	18	LYS	HB3	H	1.97
132	18	LYS	HG2	H	1.39
133	18	LYS	HG3	H	1.27
134	18	LYS	HD2	H	1.70
135	18	LYS	HD3	H	1.60
136	18	LYS	HE2	H	2.95
137	18	LYS	HE3	H	2.95
138	18	LYS	C	C	175.74
139	18	LYS	CA	C	57.85
140	18	LYS	CB	C	29.95
141	18	LYS	CD	C	27.55
142	18	LYS	CE	C	39.77
143	18	LYS	N	N	120.70
144	19	CYS	H	H	7.59
145	19	CYS	HA	H	4.20
146	19	CYS	HB2	H	3.02
147	19	CYS	HB3	H	2.95
148	19	CYS	C	C	177.01
149	19	CYS	CA	C	60.14
150	19	CYS	CB	C	24.32
151	19	CYS	N	N	116.91
152	20	LEU	H	H	8.03
153	20	LEU	HA	H	4.09
154	20	LEU	HB2	H	1.80
155	20	LEU	HB3	H	1.54
156	20	LEU	HD1	H	0.90
157	20	LEU	HD2	H	0.82
158	20	LEU	C	C	175.16
159	20	LEU	CA	C	55.39
160	20	LEU	CB	C	39.82
161	20	LEU	CD1	C	21.58
162	20	LEU	CD2	C	25.17
163	20	LEU	N	N	121.40
164	21	ARG	H	H	8.58
165	21	ARG	HA	H	3.61
166	21	ARG	HB2	H	1.95
167	21	ARG	C	C	175.45
168	21	ARG	CA	C	58.16
169	21	ARG	CB	C	27.32
170	21	ARG	N	N	118.96
171	22	TYR	H	H	7.43
172	22	TYR	HA	H	3.91
173	22	TYR	C	C	175.54
174	22	TYR	CA	C	59.04
175	22	TYR	CB	C	35.58

176	22	TYR	N	N	116.61
177	23	ARG	H	H	7.88
178	23	ARG	HA	H	4.04
179	23	ARG	HB2	H	2.04
180	23	ARG	HB3	H	2.04
181	23	ARG	HG2	H	1.70
182	23	ARG	HG3	H	1.70
183	23	ARG	HD2	H	3.26
184	23	ARG	HD3	H	3.26
185	23	ARG	C	C	176.67
186	23	ARG	CA	C	57.11
187	23	ARG	CB	C	28.01
188	23	ARG	CG	C	25.77
189	23	ARG	CD	C	41.55
190	23	ARG	N	N	119.89
191	24	LEU	H	H	8.59
192	24	LEU	HA	H	4.18
193	24	LEU	HB2	H	1.89
194	24	LEU	HB3	H	1.46
195	24	LEU	HD1	H	0.80
196	24	LEU	HD2	H	0.60
197	24	LEU	C	C	177.05
198	24	LEU	CA	C	55.00
199	24	LEU	CB	C	38.81
200	24	LEU	CD1	C	21.32
201	24	LEU	CD2	C	22.99
202	24	LEU	N	N	117.28
203	25	ARG	H	H	7.75
204	25	ARG	HA	H	4.26
205	25	ARG	HB2	H	1.91
206	25	ARG	HB3	H	1.91
207	25	ARG	HG2	H	1.82
208	25	ARG	HG3	H	1.82
209	25	ARG	HD2	H	3.11
210	25	ARG	HD3	H	3.11
211	25	ARG	C	C	177.46
212	25	ARG	CA	C	56.71
213	25	ARG	CB	C	27.46
214	25	ARG	CG	C	25.14
215	25	ARG	CD	C	41.30
216	25	ARG	N	N	120.30
217	26	LYS	H	H	7.28
218	26	LYS	HA	H	4.17
219	26	LYS	HB2	H	1.60
220	26	LYS	HB3	H	1.60
221	26	LYS	HG2	H	1.22
222	26	LYS	HG3	H	1.22
223	26	LYS	HD2	H	1.57
224	26	LYS	HD3	H	1.57
225	26	LYS	HE2	H	2.86
226	26	LYS	HE3	H	2.88
227	26	LYS	C	C	175.55
228	26	LYS	CA	C	54.84
229	26	LYS	CB	C	29.70
230	26	LYS	CG	C	22.19
231	26	LYS	CD	C	26.73
232	26	LYS	CE	C	39.22
233	26	LYS	N	N	115.77
234	27	HIS	H	H	7.82
235	27	HIS	HA	H	5.01
236	27	HIS	HB2	H	3.40

237	27	HIS	HB3	H	2.87
238	27	HIS	C	C	174.21
239	27	HIS	CA	C	52.56
240	27	HIS	CB	C	27.78
241	27	HIS	N	N	118.14
242	28	SER	H	H	7.50
243	28	SER	HA	H	3.46
244	28	SER	HB2	H	3.80
245	28	SER	HB3	H	3.80
246	28	SER	C	C	173.31
247	28	SER	CA	C	58.63
248	28	SER	CB	C	60.65
249	28	SER	N	N	114.42
250	29	ASP	H	H	8.46
251	29	ASP	HA	H	4.42
252	29	ASP	HB2	H	2.43
253	29	ASP	HB3	H	2.21
254	29	ASP	C	C	171.83
255	29	ASP	CA	C	52.93
256	29	ASP	CB	C	37.38
257	29	ASP	N	N	118.29
258	30	HIS	H	H	8.31
259	30	HIS	HA	H	4.90
260	30	HIS	HB2	H	3.75
261	30	HIS	HB3	H	3.33
262	30	HIS	C	C	175.04
263	30	HIS	CA	C	53.95
264	30	HIS	CB	C	29.17
265	30	HIS	N	N	116.46
266	31	TYR	H	H	7.05
267	31	TYR	HA	H	4.57
268	31	TYR	HB2	H	2.58
269	31	TYR	HB3	H	2.58
270	31	TYR	C	C	170.71
271	31	TYR	CA	C	54.00
272	31	TYR	CB	C	37.51
273	31	TYR	N	N	112.10
274	32	ARG	H	H	8.78
275	32	ARG	HA	H	4.24
276	32	ARG	HB2	H	1.90
277	32	ARG	HB3	H	1.90
278	32	ARG	HG2	H	0.50
279	32	ARG	HG3	H	0.50
280	32	ARG	HD2	H	2.44
281	32	ARG	HD3	H	2.25
282	32	ARG	C	C	170.17
283	32	ARG	CA	C	55.16
284	32	ARG	CB	C	27.64
285	32	ARG	CG	C	28.32
286	32	ARG	CD	C	41.50
287	32	ARG	N	N	119.90
288	33	ASP	H	H	7.55
289	33	ASP	HA	H	4.91
290	33	ASP	HB2	H	2.12
291	33	ASP	HB3	H	1.75
292	33	ASP	C	C	171.83
293	33	ASP	CA	C	49.82
294	33	ASP	CB	C	42.75
295	33	ASP	N	N	118.71
296	34	ILE	H	H	9.72
297	34	ILE	HA	H	5.41

298	34	ILE	HB	H	1.31
299	34	ILE	HG2	H	0.91
300	34	ILE	HD1	H	0.45
301	34	ILE	C	C	170.37
302	34	ILE	CA	C	57.10
303	34	ILE	CB	C	39.64
304	34	ILE	CG2	C	17.26
305	34	ILE	N	N	116.54
306	35	SER	H	H	9.53
307	35	SER	HA	H	5.10
308	35	SER	HB2	H	3.98
309	35	SER	HB3	H	3.98
310	35	SER	C	C	173.41
311	35	SER	CA	C	56.93
312	35	SER	CB	C	64.81
313	35	SER	N	N	127.07
314	36	SER	H	H	8.34
315	36	SER	HA	H	4.17
316	36	SER	HB2	H	2.94
317	36	SER	HB3	H	2.94
318	36	SER	C	C	171.93
319	36	SER	CA	C	56.27
320	36	SER	CB	C	61.52
321	36	SER	N	N	111.52
322	37	THR	H	H	8.87
323	37	THR	HA	H	4.42
324	37	THR	HB	H	3.98
325	37	THR	HG2	H	0.99
326	37	THR	C	C	172.22
327	37	THR	CA	C	61.50
328	37	THR	CB	C	66.25
329	37	THR	CG2	C	20.38
330	37	THR	N	N	118.94
331	38	TRP	H	H	9.25
332	38	TRP	HA	H	4.75
333	38	TRP	HB2	H	2.54
334	38	TRP	HB3	H	2.54
335	38	TRP	C	C	172.46
336	38	TRP	CA	C	52.15
337	38	TRP	CB	C	29.53
338	38	TRP	N	N	129.61
339	39	HIS	H	H	7.89
340	39	HIS	HA	H	4.44
341	39	HIS	HB2	H	2.43
342	39	HIS	HB3	H	2.43
343	39	HIS	C	C	169.88
344	39	HIS	CA	C	52.09
345	39	HIS	CB	C	30.38
346	40	TRP	H	H	8.56
347	40	TRP	HA	H	5.08
348	40	TRP	HB2	H	3.64
349	40	TRP	HB3	H	2.87
350	40	TRP	C	C	171.67
351	40	TRP	CA	C	53.85
352	40	TRP	CB	C	27.77
353	40	TRP	N	N	120.03
354	41	THR	H	H	8.67
355	41	THR	HA	H	4.42
356	41	THR	HB	H	3.92
357	41	THR	HG2	H	0.99
358	41	THR	C	C	175.17

359	41	THR	CA	C	62.27
360	41	THR	CB	C	67.99
361	41	THR	CG2	C	20.38
362	41	THR	N	N	115.31
363	42	GLY	H	H	9.77
364	42	GLY	HA2	H	4.03
365	42	GLY	HA3	H	4.03
366	42	GLY	C	C	173.88
367	42	GLY	CA	C	43.28
368	42	GLY	N	N	114.16
369	43	ALA	H	H	8.31
370	43	ALA	HA	H	4.32
371	43	ALA	HB	H	1.39
372	43	ALA	C	C	172.26
373	43	ALA	CA	C	50.72
374	43	ALA	CB	C	16.84
375	43	ALA	N	N	123.70
376	44	GLY	H	H	8.42
377	44	GLY	HA2	H	4.10
378	44	GLY	HA3	H	3.91
379	44	GLY	C	C	176.29
380	44	GLY	CA	C	43.25
381	44	GLY	N	N	108.16
382	45	ASN	HA	H	4.75
383	45	ASN	HB2	H	2.93
384	45	ASN	HB3	H	2.75
385	45	ASN	C	C	172.12
386	45	ASN	CA	C	50.98
387	45	ASN	CB	C	37.51
388	45	ASN	N	N	117.19
389	46	GLU	H	H	8.81
390	46	GLU	HA	H	3.98
391	46	GLU	HB2	H	1.93
392	46	GLU	HB3	H	1.87
393	46	GLU	HG2	H	2.14
394	46	GLU	HG3	H	2.14
395	46	GLU	C	C	173.36
396	46	GLU	CA	C	55.97
397	46	GLU	CB	C	27.17
398	46	GLU	CG	C	33.95
399	46	GLU	N	N	119.81
400	47	LYS	H	H	8.17
401	47	LYS	HA	H	4.19
402	47	LYS	HB2	H	1.94
403	47	LYS	HB3	H	1.76
404	47	LYS	HG2	H	1.40
405	47	LYS	HG3	H	1.33
406	47	LYS	HD2	H	1.60
407	47	LYS	HD3	H	1.60
408	47	LYS	HE2	H	2.94
409	47	LYS	HE3	H	2.94
410	47	LYS	C	C	174.43
411	47	LYS	CA	C	54.79
412	47	LYS	CB	C	30.57
413	47	LYS	CG	C	22.93
414	47	LYS	CD	C	26.73
415	47	LYS	CE	C	39.80
416	47	LYS	N	N	117.28
417	48	THR	H	H	7.49
418	48	THR	HA	H	4.37
419	48	THR	HB	H	3.99

420	48	THR	HG1	H	1.05
421	48	THR	HG2	H	1.05
422	48	THR	C	C	174.80
423	48	THR	CA	C	59.28
424	48	THR	CB	C	68.23
425	48	THR	CG2	C	19.72
426	48	THR	N	N	113.55
427	49	GLY	H	H	8.64
428	49	GLY	HA2	H	4.28
429	49	GLY	HA3	H	3.05
430	49	GLY	C	C	171.67
431	49	GLY	CA	C	42.01
432	49	GLY	N	N	111.32
433	50	ILE	H	H	8.29
434	50	ILE	HA	H	4.53
435	50	ILE	HB	H	-1.31
436	50	ILE	HG2	H	-0.31
437	50	ILE	C	C	168.12
438	50	ILE	CA	C	57.68
439	50	ILE	CB	C	37.82
440	50	ILE	N	N	119.88
441	51	LEU	H	H	8.39
442	51	LEU	HA	H	4.30
443	51	LEU	HB2	H	1.44
444	51	LEU	HB3	H	1.24
445	51	LEU	HG	H	1.44
446	51	LEU	HD1	H	0.67
447	51	LEU	C	C	171.45
448	51	LEU	CA	C	51.06
449	51	LEU	CB	C	44.03
450	51	LEU	CG	C	24.41
451	51	LEU	CD1	C	23.46
452	51	LEU	N	N	120.99
453	52	THR	H	H	8.89
454	52	THR	HA	H	5.22
455	52	THR	HB	H	3.52
456	52	THR	HG2	H	1.30
457	52	THR	C	C	173.14
458	52	THR	CA	C	59.30
459	52	THR	CB	C	72.25
460	52	THR	CG2	C	22.71
461	52	THR	N	N	120.58
462	53	VAL	H	H	8.97
463	53	VAL	HA	H	4.71
464	53	VAL	HB	H	1.65
465	53	VAL	HG1	H	0.43
466	53	VAL	HG2	H	0.16
467	53	VAL	C	C	170.60
468	53	VAL	CA	C	58.06
469	53	VAL	CB	C	31.00
470	53	VAL	CG1	C	18.20
471	53	VAL	CG2	C	20.37
472	53	VAL	N	N	127.66
473	54	THR	H	H	8.63
474	54	THR	HA	H	5.00
475	54	THR	HB	H	3.87
476	54	THR	HG2	H	1.03
477	54	THR	C	C	172.93
478	54	THR	CA	C	56.41
479	54	THR	CB	C	68.61
480	54	THR	CG2	C	19.60

481	54	THR	N	N	114.36
482	55	TYR	H	H	7.26
483	55	TYR	HA	H	4.61
484	55	TYR	HB2	H	3.55
485	55	TYR	HB3	H	3.55
486	55	TYR	C	C	171.06
487	55	TYR	CA	C	55.21
488	55	TYR	CB	C	40.88
489	55	TYR	N	N	113.74
490	56	HIS	H	H	9.34
491	56	HIS	HA	H	4.42
492	56	HIS	HB2	H	3.08
493	56	HIS	HB3	H	2.81
494	56	HIS	C	C	173.18
495	56	HIS	CA	C	56.49
496	56	HIS	CB	C	29.81
497	56	HIS	N	N	118.21
498	57	SER	H	H	7.34
499	57	SER	C	C	173.49
500	57	SER	CA	C	54.41
501	57	SER	N	N	105.78
502	59	THR	HA	H	3.91
503	59	THR	HB	H	4.07
504	59	THR	HG2	H	1.20
505	59	THR	CA	C	64.19
506	59	THR	CB	C	66.34
507	59	THR	CG2	C	18.99
508	60	GLN	H	H	8.02
509	60	GLN	HA	H	4.06
510	60	GLN	HB2	H	2.09
511	60	GLN	HB3	H	2.09
512	60	GLN	HG2	H	3.26
513	60	GLN	HG3	H	3.26
514	60	GLN	C	C	174.20
515	60	GLN	CA	C	56.90
516	60	GLN	CB	C	27.27
517	60	GLN	CG	C	41.55
518	60	GLN	N	N	123.81
519	61	ARG	H	H	7.31
520	61	ARG	HA	H	2.99
521	61	ARG	HB2	H	1.70
522	61	ARG	HB3	H	1.70
523	61	ARG	C	C	175.22
524	61	ARG	CA	C	57.25
525	61	ARG	CB	C	27.77
526	61	ARG	N	N	119.25
527	62	THR	H	H	8.47
528	62	THR	HA	H	3.71
529	62	THR	HB	H	4.21
530	62	THR	HG2	H	1.16
531	62	THR	C	C	174.94
532	62	THR	CA	C	64.67
533	62	THR	CB	C	66.46
534	62	THR	CG2	C	19.65
535	62	THR	N	N	117.57
536	63	LYS	H	H	7.88
537	63	LYS	HA	H	4.05
538	63	LYS	HB2	H	1.90
539	63	LYS	HB3	H	1.90
540	63	LYS	HG2	H	1.29
541	63	LYS	HG3	H	1.29

542	63	LYS	HD2	H	1.59
543	63	LYS	HD3	H	1.59
544	63	LYS	HE2	H	2.84
545	63	LYS	HE3	H	2.79
546	63	LYS	C	C	173.47
547	63	LYS	CA	C	57.28
548	63	LYS	CB	C	29.34
549	63	LYS	CG	C	22.63
550	63	LYS	CD	C	26.76
551	63	LYS	CE	C	39.80
552	63	LYS	N	N	121.56
553	64	PHE	HA	H	3.94
554	64	PHE	HB2	H	3.75
555	64	PHE	HB3	H	3.75
556	64	PHE	C	C	177.53
557	64	PHE	CA	C	59.77
558	64	PHE	CB	C	35.86
559	64	PHE	N	N	122.19
560	65	LEU	H	H	8.46
561	65	LEU	HA	H	4.03
562	65	LEU	HB2	H	1.92
563	65	LEU	HB3	H	1.33
564	65	LEU	HD1	H	0.67
565	65	LEU	HD2	H	0.48
566	65	LEU	C	C	174.91
567	65	LEU	CA	C	54.86
568	65	LEU	CB	C	39.32
569	65	LEU	CD1	C	19.30
570	65	LEU	CD2	C	22.91
571	65	LEU	N	N	118.84
572	66	ASN	H	H	7.89
573	66	ASN	HA	H	4.72
574	66	ASN	HB2	H	2.84
575	66	ASN	HB3	H	2.76
576	66	ASN	C	C	176.34
577	66	ASN	CA	C	51.67
578	66	ASN	CB	C	37.26
579	66	ASN	N	N	114.93
580	67	THR	H	H	7.52
581	67	THR	HA	H	4.25
582	67	THR	HB	H	3.74
583	67	THR	HG2	H	0.96
584	67	THR	C	C	173.66
585	67	THR	CA	C	61.85
586	67	THR	CB	C	68.91
587	67	THR	CG2	C	18.92
588	67	THR	N	N	112.40
589	68	VAL	H	H	7.73
590	68	VAL	HA	H	3.39
591	68	VAL	HB	H	1.05
592	68	VAL	HG1	H	0.16
593	68	VAL	HG2	H	-0.12
594	68	VAL	C	C	171.61
595	68	VAL	CA	C	60.07
596	68	VAL	CB	C	29.25
597	68	VAL	CG1	C	18.45
598	68	VAL	CG2	C	17.60
599	68	VAL	N	N	122.00
600	69	ALA	H	H	8.12
601	69	ALA	HA	H	4.23
602	69	ALA	HB	H	1.19

603	69	ALA	C	C	172.02
604	69	ALA	CA	C	49.53
605	69	ALA	CB	C	15.99
606	69	ALA	N	N	129.17
607	70	ILE	H	H	8.40
608	70	ILE	C	C	174.04
609	70	ILE	CA	C	54.26
610	70	ILE	N	N	125.89
611	71	PRO	HA	H	4.43
612	71	PRO	HB3	H	1.92
613	71	PRO	HG2	H	3.83
614	71	PRO	HG3	H	3.35
615	71	PRO	CA	C	60.85
616	71	PRO	CB	C	30.38
617	71	PRO	CG	C	25.23
618	72	ASP	H	H	8.56
619	72	ASP	HA	H	4.19
620	72	ASP	HB2	H	2.65
621	72	ASP	HB3	H	2.65
622	72	ASP	C	C	174.61
623	72	ASP	CA	C	53.85
624	72	ASP	CB	C	38.07
625	72	ASP	N	N	120.03
626	73	SER	H	H	7.48
627	73	SER	HA	H	4.26
628	73	SER	HB2	H	4.07
629	73	SER	HB3	H	3.83
630	73	SER	C	C	173.98
631	73	SER	CA	C	55.90
632	73	SER	CB	C	60.58
633	73	SER	N	N	109.69
634	74	VAL	H	H	7.83
635	74	VAL	HA	H	4.45
636	74	VAL	HB	H	1.99
637	74	VAL	HG1	H	0.66
638	74	VAL	HG2	H	0.62
639	74	VAL	C	C	171.92
640	74	VAL	CA	C	59.08
641	74	VAL	CB	C	30.98
642	74	VAL	CG1	C	20.02
643	74	VAL	CG2	C	20.02
644	74	VAL	N	N	125.42
645	75	GLN	H	H	8.94
646	75	GLN	HA	H	4.45
647	75	GLN	HB2	H	2.03
648	75	GLN	HB3	H	1.90
649	75	GLN	HG2	H	2.43
650	75	GLN	HG3	H	2.23
651	75	GLN	C	C	172.04
652	75	GLN	CA	C	53.00
653	75	GLN	CB	C	28.74
654	75	GLN	CG	C	32.19
655	75	GLN	N	N	125.65
656	76	ILE	H	H	8.83
657	76	ILE	HA	H	4.63
658	76	ILE	HB	H	1.88
659	76	ILE	HG2	H	0.67
660	76	ILE	C	C	172.76
661	76	ILE	CA	C	58.71
662	76	ILE	CB	C	37.76
663	76	ILE	CG2	C	15.81

664	76	ILE	N	N	122.43
665	77	LEU	H	H	9.07
666	77	LEU	HA	H	5.04
667	77	LEU	HB2	H	1.65
668	77	LEU	HB3	H	1.30
669	77	LEU	HG	H	1.43
670	77	LEU	HD1	H	0.74
671	77	LEU	HD2	H	0.60
672	77	LEU	C	C	172.98
673	77	LEU	CA	C	51.54
674	77	LEU	CB	C	41.98
675	77	LEU	CG	C	25.94
676	77	LEU	CD1	C	22.69
677	77	LEU	CD2	C	22.12
678	77	LEU	N	N	128.16
679	78	VAL	H	H	8.87
680	78	VAL	HA	H	4.38
681	78	VAL	HB	H	1.55
682	78	VAL	HG1	H	0.71
683	78	VAL	HG2	H	0.71
684	78	VAL	C	C	173.14
685	78	VAL	CA	C	58.45
686	78	VAL	CB	C	32.33
687	78	VAL	CG1	C	19.09
688	78	VAL	CG2	C	19.09
689	78	VAL	N	N	121.05
690	79	GLY	H	H	7.86
691	79	GLY	HA2	H	5.08
692	79	GLY	HA3	H	4.08
693	79	GLY	C	C	172.86
694	79	GLY	CA	C	44.62
695	79	GLY	N	N	111.73
696	80	TYR	H	H	8.54
697	80	TYR	HA	H	5.37
698	80	TYR	HB2	H	2.99
699	80	TYR	HB3	H	2.61
700	80	TYR	C	C	169.75
701	80	TYR	CA	C	54.23
702	80	TYR	CB	C	40.30
703	80	TYR	N	N	119.24
704	81	MET	H	H	8.60
705	81	MET	HA	H	5.35
706	81	MET	HB2	H	1.94
707	81	MET	HB3	H	1.94
708	81	MET	HG2	H	2.55
709	81	MET	HG3	H	2.50
710	81	MET	C	C	171.31
711	81	MET	CA	C	51.86
712	81	MET	CB	C	34.66
713	81	MET	CG	C	29.09
714	81	MET	N	N	117.15
715	82	THR	H	H	8.53
716	82	THR	HA	H	4.98
717	82	THR	HB	H	3.51
718	82	THR	HG2	H	1.06
719	82	THR	C	C	172.03
720	82	THR	CA	C	59.38
721	82	THR	CB	C	68.52
722	82	THR	CG2	C	19.60
723	82	THR	N	N	122.12
724	83	MET	H	H	8.25

725	83	MET	HA	H	5.19
726	83	MET	C	C	170.95
727	83	MET	CA	C	51.06
728	83	MET	CB	C	33.27
729	83	MET	N	N	122.01
730	84	HIS	H	H	8.90
731	84	HIS	C	C	173.02
732	84	HIS	CA	C	53.04
733	84	HIS	N	N	118.65